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In silico and DNA photocleavage studies of *N, N`-bis*(1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)malonohydrazide Schiff's base and its metal complexes

R. Pal[1], V. Kumar[1*], V. Beniwal[2], G. K. Gupta[3] and A. K. Gupta[1]*

[1]*Department of Chemistry, Maharishi Markandeshwar University, Mullana, Ambala, India*
[2]*Department of Biotechnology, Maharishi Markandeshwar University, Mullana, Ambala, India*
[3]*Maharishi Markandeshwar College of Pharmacy, Maharishi Markandeshwar University, Mullana, Ambala, India*

ABSTRACT

A series of metal complexes of Cu(II), Ni(II), Co(II), Mn(II), Zn(II) with a tetradentate N,N`-bis(1-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene)malonohydrazide Schiff's base has been synthesized under solvent free conditions. Structures of the compounds were confirmed on the basis of spectroscopic data. All compounds were screened for their DNA photocleavage ability and it has been found that metal complexes showed good DNA cleaving properties. Among them Cu(II) complex acts as a potential nuclease agent as compared to ligand and other synthesized metal complexes. To explore the further biological potential on the basis of interactions between DNA gyrase and ligand in silico study was carried out using Molegro Virtual Docker.

Key words: DNA photocleavage, docking study, dehydroacetic acid, solvent free synthesis

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INTRODUCTION

Owing to excellent chelating behavior Schiff's base chemistry has exclusively been studied. They possessed a wide range of pharmaceutical applications [1, 2] and biological activities [3, 4]. Moreover, the presence of -CO-NHN=C- pharmacophore is known to be responsible for the biological activity of hydrazone Schiff's bases [5]. As a result, compounds having this structural feature represent an important class of pharmaceutical and medicinal agents [6-8]. Various Schiff's bases derived from dehydroacetic acid and their complexes with various metal ions have been reported to exhibit antimicrobial activity [9-11]. In recent years, more attention was paid to evaluate the DNA cleavage potential of Schiff metal complexes due to the successful clinical applications of cisplatin in cancer chemotherapy [12]. Amongst them, transition metal complexes particularly are known as good DNA nicking agents and Cu(II) complexes specifically are found to possess excellent DNA cleaving properties [13] due to their ability to bind with the DNA molecule. As DNA is the primary site where most of chemotherapeutic drugs act and result the cleavage or damage of DNA structure which further leads to the inhibition or death of cancerous cells [14]. In spite of their huge applications in different fields novel synthetic routes are always in demand.

In recent years, area of non conventional methods of synthesis such as microwave assisted synthesis, grind stone technique and ultrasound irradiation has been explored well due to less reaction time, good yield, no use of

hazardous solvents and easy work [15-18]. Keeping these observations in mind and to explore the potential of some novel compounds, in the present investigation, we report a solvent free synthesis of hydrazone Schiff base metal complexes of Cu(II), Ni(II), Mn(II), Co(II) and Zn(II). Furthermore, an effort has been made to understand the ligand-receptor interactions based on bacterial DNA gyrase as a target enzyme using Molegro Virtual Docker (MVD) with an expectation to find some new potential DNA nicking and antibacterial agents..

MATERIALS AND METHODS

DHA was purchased from Merck and used without further purification. All other chemicals including solvents were of LR grade and used as supplied. Double distilled water was used in the present investigation. The ^{1}H and ^{13}C NMR spectra of the ligand and complexes were recorded on Bruker 400 MHz instrument. IR spectra were measured on Shimadzu IR Affinity in 4000 to 400 cm^{-1} range using KBr pellet method. The electronic spectra were recorded using Shimadzu UV 1800 instrument in DMSO as a solvent. Mass spectra were recorded on Agilent Mass Spectrometer. The three-dimensional (3D) chemical structures were drawn by using the Structure Builder (ACD Chem-Sketch 8.0, ACD Labs, Toronto). Docking studies were carried out by using the facilities of Molecular Modelling (Molegro Virtual Docker 2010.4.2.0, Molegro Bioinformatics, Aarchus C, Denmark).

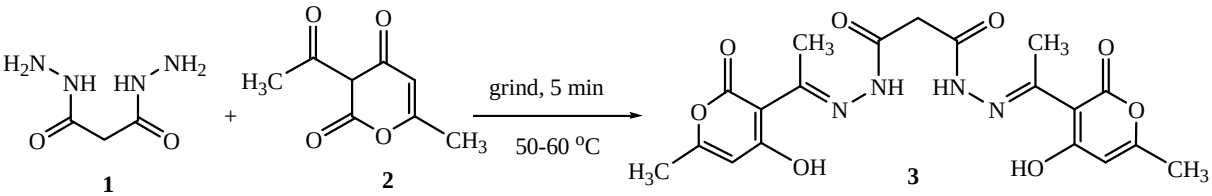
Synthesis of malonyl dihydrazide

Malonyl dihydrazide was prepared by treating diethyl malonate (0.01 mol) and hydrazine hydrate (0.02 mol) according to the literature procedure [19].

Green synthesis of ligand (3)

A mixture of malonyl dihydrazide (0.01 mol) and DHA (0.02 mol) was ground quickly for 5 minutes with the help of pestle in a mortar at 50-60 °C temperature. The reaction mass first was converted into homogeneous liquid and finally converted into solid mass. The progress of reaction was monitored by TLC. After completion, the reaction mass was poured into chilled water and solid product thus obtained was filtered, washed with 70 % aqueous ethanolic solution and dried in hot air oven at 65 °C for 4 h.

Scheme 1. Green synthesis of the hydrazone Schiff's base ligand



Green synthesis of metal complexes

General procedure:

A mixture of ligand and appropriate metal salt in a pre heated mortar at 80 °C was ground quickly for 3-6 min at the same temperature. The complexes thus obtained were washed with ethanol followed by petroleum ether wash to remove traces of unreacted metal salts and were dried in hot air oven at 70 °C for 5 h.

Spectral data of some selected compounds

N,N`-*bis*(1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene)malonohydrazide (3): m.p. 225 °C; Mass; 433.1; IR (KBr cm^{-1}): 3,410 (O-H str.), 1,659(C=N str.), 1,692(C=O str.), 1,261(C-O str.); ^{1}H NMR (ppm): 2.14 (s, 3H of C**H**$_3$-), 2.57 (s, 3H of -N=C-C**H**$_3$), 3.5 (s, 2H of COC**H**$_2$CO), 5.83 (s, 1H of DHA ring), 11.45 (s,1H of -CON**H**-), 16.2 (s, 1H of enolic proton). ^{13}C NMR (ppm): 16.7 (CH_3 of DHA ring), 19.2 (-N=C-CH_3), 162 (-N=C-CH_3), 94.7 (CO**C**H$_2$CO), 168 (**C**OCH$_2$**C**O), 180 (enolic C), 163 (carbonyl carbon of DHA ring), Yield 79%.

N,N`-*bis*(1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene)malonohydrazide Co complex: m.p. 265°C; Mass; 489.2; IR (KBr cm^{-1}): 3,421(O-H str.), 1,635(C=N), 1,673(C=O str.), 1,246(C-O str.), 460(M-N), 639(M-O); Yield 82%.

N,N`-*bis*(1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene)malonohydrazide Cu complex: m.p. 267°C; Mass; 494.0; IR (KBr cm^{-1}): 3,405(O-H str.), 1,658 (C=N), 1,691(C=O str.), 1,285 (C-O str.), 478 (M-N), 643 (M-O); Yield 89%.

N,N`-*bis*(1-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)ethylidene)malonohydrazide Zn complex: m.p. 298°C; Mass; 496.1; IR (KBr cm^{-1}): 3,422(O-H str.), 1,628(C=N), 1,673(C=O str.), 1,271(C-O str.), 486(M-N), 637(M-O); ^{1}H NMR (ppm): 2.04 (s, 3H of C**H**$_3$-), 2.55 (s, 3H -N=C-C**H**$_3$), 3.24 (s, 2H of COC**H**$_2$CO), 5.57 (s, 1H of DHA ring), 11.75 (s,1H of -CON**H**-); Yield 87%.

***In Silico* study**

The ligand structure was constructed using Chem-Sketch software (ACD Labs) and saved in MOL format. The protein structure of DNA gyrase subunit A Escherichia coli (PDB Code: 1AB4) was imported in MVD, and missing bond orders, hybridization states, and angles then were assigned. To obtain better potential binding sites in the protein, maximum of five cavities were detected using parameters such as molecular surface (expanded van der Waals), maximum number of cavities (n = 5), minimum cavity volume (10), the probe size (1.20), the maximum amount of ray checks (n = 16), minimum number of ray hits (n = 12), and grid resolution (0.80). The setup for side chain flexibility by selection of the add-visible option, the setting for the selected flexible side chain during the docking option, and all other parameters were kept in default. All docking calculations were carried out using the grid-based Mol Dock score (GRID) function with a grid resolution of 0.30 A$^{\circ}$. The Mol Dock optimization search algorithm with a maximum of 10 runs was used through the calculations, with all other parameters kept as defaults. All the poses were examined manually, and the best pose was retained.

DNA photocleavage studies

DNA photocleavage experiment was performed in a volume of 10 µl containing plasmid DNA in TE (*Tris*, 10mM, EDTA 0.01mM, pH 8.0) buffer in the presence of 40 µg of synthesized compounds. The reaction volumes were held in caps of polyethylene microcentrifuge tubes, which were placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm. The samples were irradiated for 30 min at room temperature and were further incubated at 37 $^{\circ}$C for 1 h. Irradiated samples were mixed with 6X loading dye containing 0.25% bromophenol blue and 30% glycerol and then were analyzed by electrophoresis on a 0.8% agarose horizontal slab gel in *Tris*-acetate EDTA buffer (40 mM *Tris*, 20 mM acetic acid, 1 mM EDTA, pH: 8.0). Untreated plasmid DNA was maintained as a control in each run of gel electrophoresis, which was carried out at 5V/cm for 2.0 h. The gel was stained with ethidium bromide (1 µg/ml) and photographed under UV light.

RESULTS AND DISCUSSION

In continuation of our ongoing interest to develop the convenient and efficient methodologies towards the synthesis of biologically active compounds [20-22], herein we report the solvent free synthesis of heterocyclic hydrazone Schiff base and its complexes with first transition metal series. In the conventional approach, entitled compound **(3)** was prepared by reacting malonyl dihydrazide with DHA in 50 ml of aqueous ethanol under reflux for 1h. However, in solvent free approach reactions were completed in 1-6 min and gave desired products with good yields and purity. Therefore, the present method has many advantages over conventional methods like no solvent requirement, simplicity, easy operation, eco friendly and less reaction time with better yields of desired products. The structure of all the synthesized compounds were characterized on the basis of ^{1}H and ^{13}C NMR, UV-visible, mass spectrometry.

^{1}H NMR

In the ^{1}H NMR spectrum of metal complexes enolic singlet of the ligand (**3**) was found to be disappeared which suggested the deprotonation of enolic oxygen prior to complexation. Shifting of chemical shifts of the ligand's protons on complexation further indicated the formation of complexes.

FT-IR

The FT-IR spectrum of the ligand illustrated the characteristic bands at 3,000-3,500, 1,692, 1,659, 1,327-1,356 and 1,209-1,261 cm^{-1} which were assigned to υ_{OH} (intramolecular hydrogen bonding), $\upsilon_{C=O}$ (carbonyl group), $\upsilon_{C=N}$ (azomethine) and υ_{C-O} (enolic) stretching modes, respectively [23]. In the spectra of metal complexes the absence of a weak broad band in the range 3,300-3,500 cm^{-1} and increase in frequency of enolic C-O stretching up to 10-15 cm^{-1} suggested the deprotonation of enolic -OH occur prior to coordination. The $\upsilon_{C=N}$ shifted to lower frequency in FTIR spectra of metal complexes indicated its role in coordination with metal ions [24]. It may be due to the

donation of the lone pair of electrons of nitrogen atom of azomethine to the empty *d* orbitals, of metal ions. FTIR spectra of metal complexes showed characteristic non ligand bands in 640-670 cm^{-1} and 460-491 cm^{-1} region which can be assigned to υ_{M-N} and υ_{M-O} vibrations, respectively [25].

The electronic spectral data of the ligand as well as its metal complexes were recorded in 10^{-4} M DMSO solution.

UV-visible spectrum

The UV-visible spectrum of a free ligand showed the bands at 34482, 31250, 27472 cm^{-1}. These absorption bands were appeared due to π- π* and n- π* of carbonyl and azomethine moiety [19]. The bands due to d-d transitions at 15432 cm^{-1}, 17064 cm^{-1} and at 21008 cm^{-1} along with 16611 cm^{-1} were observed due to Cu(II), Ni(II) and Co(II) respectively.

Mass

Appearance of an intense molecular ion peak at *m/z* 433.2 (M^+ +1) in mass spectrum of the ligand corresponds to the molecular formula of ligand. The mass spectrum of Ni(II) complex showed a molecular ion peak at *m/z* 489.2. The mass spectra of Co(II), Mn(II) and Zn(II) showed an intense molecular ion peak at *m/z* 489.2, 486.3 and 496.0, respectively. Similarly, the mass spectrum of copper complex exhibited a molecular ion peak at *m/z* 494.

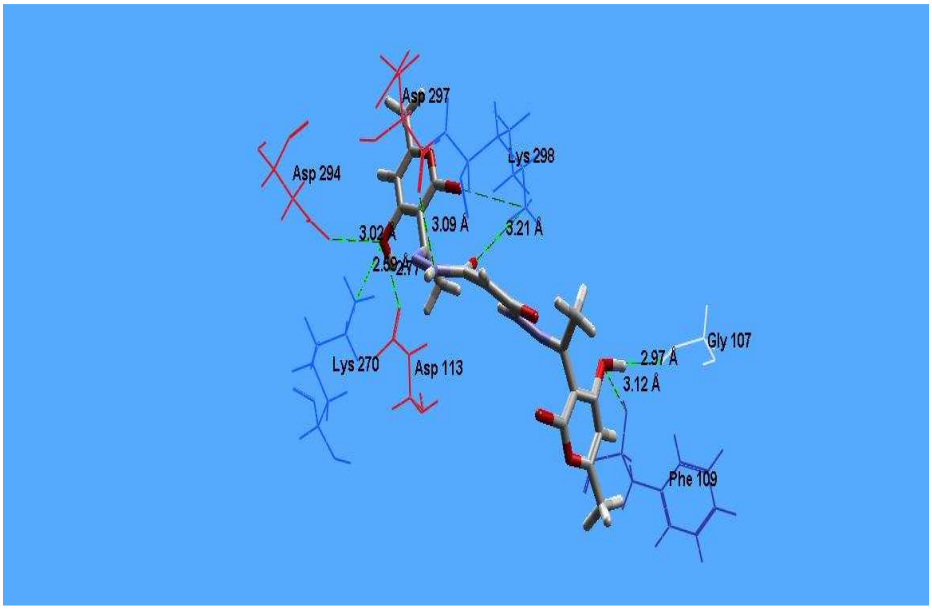


Figure 1. Docked structure of the ligand

Docking study

To obtain better potential binding sites in the protein (1AB4), maximum of five cavities was detected using default parameters. Among cavities, cavity number 1 (cavity volume, 123,904 A°) was selected for further studies. The chosen cavity was further refined using side chain minimization by selection of add visible option set at a maximum of 10,000 steps per residue and at a maximum of 10,000 global steps. The initial energy was set at 535,059, whereas the energy after side chain minimization was set at 373,048. The side chain flexibility was set by selecting the add-visible option. The same was selected during docking, and the remaining parameters were kept as fixed variables. Furthermore, the docking simulation was run first for the parent molecule [26], and the best pose was selected on the basis of the Mol Dock score and re-rank score. The best pose revealed the docking score -58.07 and form seven interactions as shown by green dotted lines (**Figure 1**), due to three hydrogen bonds between O of OH group with Lys270, Asp113, and Asp294 of distances 2.59 Å, 2.77 Å, and 3.02 Å, respectively. Two more weak hydrogen bonding, one between O of C=O of pyrone with Lys 298 of distance 3.11Å, and another between O of CONH with Lys 298 of distance 3.21 Å also play role in binding affinity. Second O of OH group of ligand also showed strong hydrogen bonding with protein residues Gly107 and Phe109 of distances 2.97 Å and 3.12 Å, respectively. The performance of the obtained pose showed that the hydrogen bond interaction between the protein residues at

Gly107, Lys270, and Asp113 and the oxygen atom of (OH) of the ligand showed maximum affinity, indicating that the docking method was most appropriate for clarifying the binding mode of the proposed compound as DNA gyrase inhibitors .

Plasmid DNA photocleavage studies

DNA photocleavage studies were performed using agarose gel electrophoresis method and results are presented in **figure 2.** There is a significant decrease in the intensity of DNA band in case of test compounds as compared with control DNA which may indicate the fragmentation caused by the test compounds; ligand (Lane 1) showed little or no fragmentation under experimental conditions. In case of Co(II), and Zn(II) complexes (Lane 3, and 7) there is a decrease in the intensity of form I and II as compared with control (C) which indicates the fragmentation induced in DNA forms by these complexes. In case of Ni(II) complex, there is an increase in intensity of form II and decrease in the intensity of form I suggest the conversion of super coiled DNA (form I) into open coiled DNA (form II) due to fragmentation caused by Ni(II) complex. In lane 5 Cu(II) complex increases the intensity of form II and is responsible for disappearance of form I i.e. super coiled DNA. Moreover, appearance of form III in between form I and II represents a linear form generated due to nicking of super coiled DNA. All the above mentioned observations showed that Cu(II) complex exhibits good DNA photocleavage as compared to ligand as well as other complexes considered under the study.

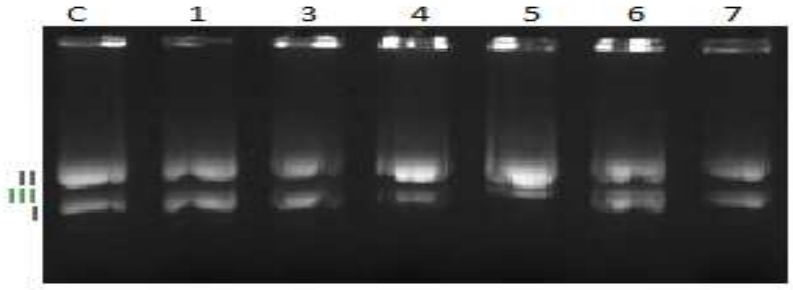


Figure 2. DNA photocleavage activity of synthesized compounds

Lane 1st (C), DNA + DMSO; lane 2nd, ligand + DNA; lane 3rd, Co(II) complex + DNA; lane 4th, Ni(II) complex + DNA; lane 5th, Cu(II) complex + DNA; lane 6th, Mn(II) complex + DNA; lane 7th, Zn(II) complex + DNA.

CONCLUSION

In search of some biologically active agents, a tetradentate heterocyclic hydrazone Schiff base (**3**) and its metal complexes of the first transition series have been designed and synthesized under solvent free conditions. All compounds were evaluated for their DNA photocleavage ability as well as DNA gyrase inhibition tendency with an expectation to find efficient DNA nicking and antibacterial agents. It has been observed that Cu(II) and Ni(II) complexes were found to exhibit good DNA fragmentation potential as compared to ligand and other complexes. The docking of ligand with DNA gyrase indicated the seven strong interactions among them, hence suggested good level of DNA gyrase inhibiting ability of ligand. Some structural modifications in the ligand may lead to development of better DNA nicking agents in future.

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